

Is biocontrol efficacy rather driven by the plant or the antagonist genotypes? A conceptual bioassay approach

Yan Sun¹, Carine Beuchat¹, Heinz Müller-Schärer¹

¹ Department of Biology/Ecology & Evolution, University of Fribourg, 1700 Fribourg, Switzerland

Corresponding author: Yan Sun (yan.sun@unifr.ch)

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Abstract

In the new range, invasive species lack their specialist co-evolved natural enemies, which then might be used as biocontrol agents. Populations of both a plant invader in the introduced range and its potential biocontrol agents in the native range may be genetically differentiated among geographically distinct regions. This, in turn, is expected to affect the outcome of their interaction when brought together, and by this the efficacy of the control. It further raises the question, is the outcome of such interactions mainly driven by the genotype of the plant invader (some plant genotypes being more resistant/tolerant to most of the antagonist genotypes), or by the antagonist genotype (some antagonist genotypes being more effective against most of the plant genotypes)? This is important for biocontrol management, as only the latter is expected to result in more effective control, when introducing the right biocontrol agent genotypes. In a third scenario, where the outcome of the interaction is driven by a specific plant by antagonist genotype interactions, an effective control will need the introduction of carefully selected multiple antagonist genotypes. Here, we challenged in a complete factorial design 11 plant genotypes (mainly half-siblings) of the invasive *Ambrosia artemisiifolia* with larvae of eight genotypes (full-siblings) of the leaf beetle *Ophraella communa*, a potential biocontrol insect, and assessed larval and adult performance and leaf consumption as proxies of their expected impact on the efficacy of biological control. Both species were collected from several locations from their native (USA) and introduced ranges (Europe and China). In summary, we found *O. communa* genotype to be the main driver of this interaction, indicating the potential for at least short-term control efficacy when introducing the best beetle genotypes. Besides the importance of investigating the genetic structure both among and within populations of the plant invader and the biocontrol agent during the pre-release phase of a biocontrol program, we advocate integrating such bioassays, as this will give a first indication of the probability for an – at least – short- to mid-term efficacy when introducing a potential biocontrol agent, and on where to find the most efficient agent genotypes.

Keywords

Ambrosia artemisiifolia, classical biological control, co-evolution, common ragweed, $G \times G$ interactions, *Ophraella communa*

Introduction

Plant-antagonist interactions are intensively studied because they influence a wide variety of ecosystems. Studying the arms-race between a plant and its enemies often focuses on herbivores selecting for plant defense traits, which in turn, select for traits in the herbivore to overcome the defense (Kareiva 1999; Rausher 2001; Taggar and Arora 2017). Plant-antagonist interactions are among the most studied evolutionary interactions (Gloss et al. 2019; Ohgushi 2016). Genetic variation within a plant species has been shown to have a strong influence on the performance of the associated herbivores for survival, developmental time and herbivore dynamics, due to differences in secondary compounds and nutrients within-plant species (Beck et al. 2014; Müller et al. 2006; Underwood and Rausher 2000). Similarly, within-species genetic variability in insects is known to differ among distinct geographic populations (Carter et al. 2009; Molfini et al. 2018; Nishide et al. 2015), which may result in different herbivore performance on their host plants (Goolsby et al. 2006; Lommen et al. 2017b). Plant-antagonist interactions are thus often genotype-specific (Burdon et al. 1996; Campanella et al. 2009). Outcomes of such a plant-antagonist arms-race have mainly been studied in agroecosystems, most prominently in crop-pest interactions (Scott et al. 2010), where crops have been selected to resist pest attacks and antagonists have overcome such defense mechanisms, often leading to the formation of biotypes of crop pathogens and pest insects (Goolsby et al. 2006). In this context, biotypes have been defined as populations within an arthropod species that show variations in their ability to effectively use a trait deployed by a plant cultivar (Taggar and Arora 2017). For instance, Zytynska and Preziosi (2011) showed that different aphid genotypes exhibited differential preference and performance (reproductive rate) for different barley genotypes and such genetic associations between the aphids and barley could lead to population-level changes within the aphid species.

Besides being relevant for agriculture, studies on plant-antagonist interactions are also crucial for interactions between a plant invader and its biological control agents (BCAs) (Müller-Schärer and Schaffner 2020; Müller-Schärer et al. 2004). Invasions are generally characterized by significant demographic events including population bottlenecks, hybridization, multiple colonization, admixtures, founder effects, and range expansion, as well as by post introduction evolutionary changes, all of which influence the amount of genetic variation and differentiation both within and among invading populations (Atwood and Meyerson 2011; Genton et al. 2005; Hodgins and Rieseberg 2011; Müller-Schärer et al. 2020; Prentis et al. 2008; Sun and Roderick 2019). Classical biological control, by importing specialist antagonists from the native

range of the plant invader has, been a most effective and sustainable control method against invasive species (Hinz et al. 2020; Müller-Schärer and Schaffner 2008; Schwarzländer et al. 2018).

Significant differences among the population in BCAs in the native range have been well documented, especially for ecological traits linked to climate and host plant use (Fukano et al. 2016; Hopper et al. 1993; Mathenge et al. 2010). This offers a multitude of genetic interactions with deliberately released BCAs and stimulated the discussion on whether single vs. multiple antagonist genotypes and populations should be introduced (multiple provenance introductions) (DeBach and Rosen 1991; Hufbauer et al. 2004). At first sight, releasing a high genetic variation of the BCA may be the best option for all cases to allow for local adaptation to environmental conditions in the introduced range. However, there are constraints and cases of potential disadvantages when using this approach. In the past, BCAs were collected from distinct populations and subsequently combined in order to increase the genetic diversity and thus to increase establishment and to promote post-release adaptation. This may enhance the control effect (DeBach and Rosen 1991; Mathenge et al. 2010), but also the risk of non-target effects (Szűcs et al. 2019). Modern guidelines for biological weed control, therefore, no longer allow this practice and further insist that single populations are assessed separately for potential efficacy and safety before their introduction, with a reference collection made of individuals from the population released (Szűcs et al. 2014; USDA-APHIS 2016). Furthermore, intraspecific hybridization among BCA populations when introducing genotypes from different populations may result in increased efficacy through heterosis, but may also reduce the genetic integrity of the best genotypes (Roderick et al. 2012). Hoffmann et al. (2002) showed that the performance of two biotypes of the cochineal scale *Dactylopius opuntiae*, each specializing on a different species of *Opuntia*, was altered in the F1 and F2 generation crosses and this may have diminished the agent's effectiveness in South Africa.

Here, we explored the genotype by genotype (G by G) interaction between the invasive alien plant *Ambrosia artemisiifolia* (*Ambrosia* in the following) and its natural enemy and potential BCA *Ophraella communa* LeSage (Coleoptera: Chrysomelidae) (*Ophraella* in the following), both native to North America and accidentally introduced into various regions worldwide (Müller-Schärer and Schaffner 2020; Müller-Schärer et al. 2018; Schaffner et al. 2020). Based on molecular marker, genomic scans and phenotyping in common environments, *Ambrosia* populations were found to greatly differ both among regions and among populations within regions (Sun and Roderick 2019; van Boheemen et al. 2019). *Ophraella* has recently and accidentally been introduced and was first recorded in China in 2001 and Europe in 2013 (Ma et al. 2008; Müller-Schärer et al. 2014). The few published studies on genetic differentiation among *Ophraella* populations indicate clear differences among regions and among populations within regions, revealed both by molecular markers and phenotyping (Bordeyne et al. 2020; Bouchemousse et al. 2020; Nishide et al. 2015).

To explore G by G interactions between *Ambrosia* and *Ophraella*, we used different parental families (full-sibs) of *Ophraella* and *Ambrosia* (half-sibs), thus representing

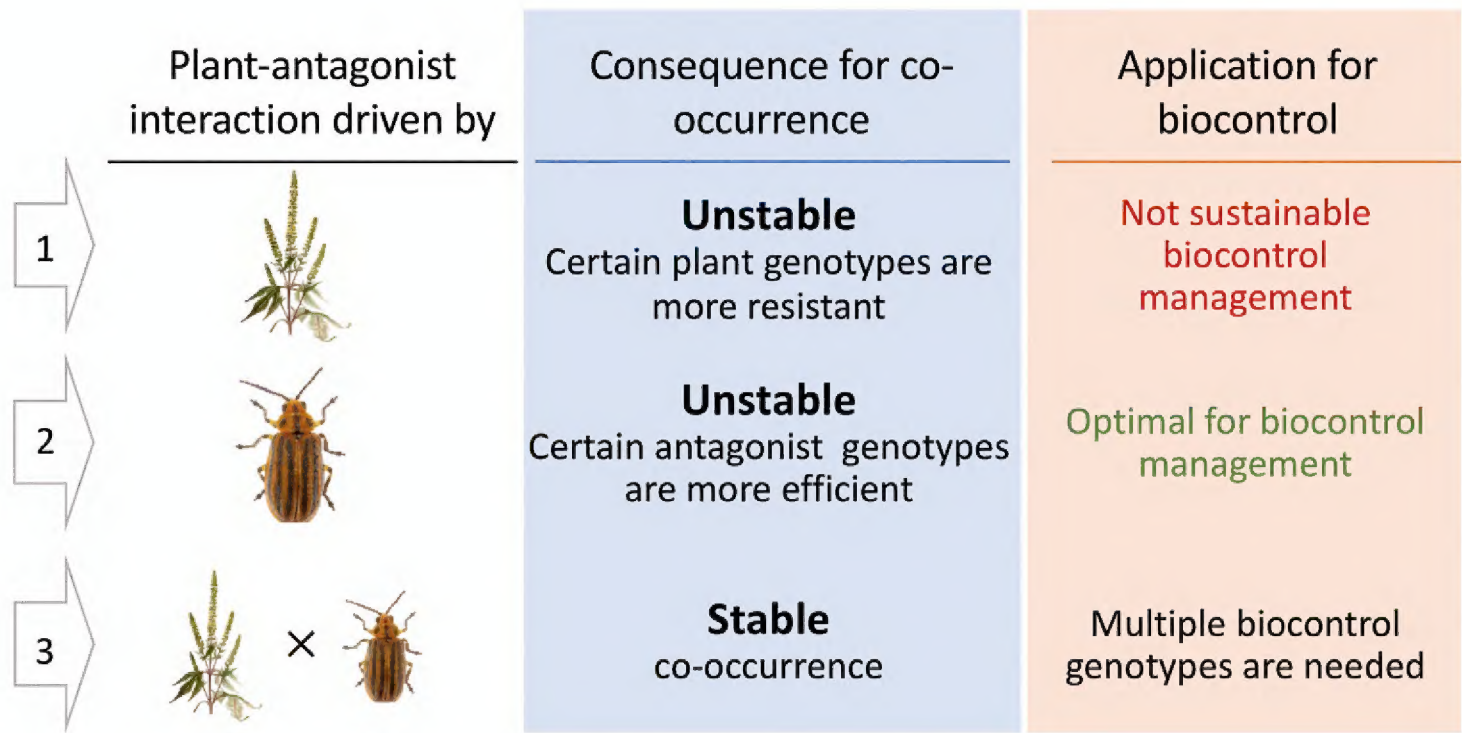


Figure 1. Three scenarios of plant-antagonist genotype interactions and their expected outcome for the biocontrol management: the performance of the antagonist is driven **1** mainly by the plant genotype **2** mainly by the antagonist genotype and **3** by the plant by antagonist genotype interactions.

genotypes. To ensure a high genetic differentiation among the genotypes of both species, we used individuals from several populations from their native (USA) and introduced ranges (Europe and China). This approach is also applicable for variation at the strain (a population arising from a single collection or clonal individual (Granett et al. 2001) or biotype level, especially if the variation of host plant use or in functional traits are known or suspected for the BCA in the native range or from the target weed in the introduced range, respectively. We assessed both *Ophraella* performance and leaf consumption as proxies of the expected impact on the efficacy of biological control (Lommen et al. 2017b). Insect performance or plant resistance is often tested by analyzing developmental time, weight, survival (Liu et al. 2012) and herbivore damage (Stenberg and Muola 2017). These vital rates are important for a BCA’s population density, and ultimately impact on the target plant invader (Augustinus et al. 2020). For instance, faster developmental time allows more generations per year and a higher survival rate would directly result in higher population growth (Augustinus et al. 2020), thus both will contribute to increased feeding damage and, together with leaf area removed (O’Neal et al. 2002; Siemann and Rogers 2003) to increased biocontrol efficacy (Gassmann 1996; Lommen et al. 2017b). Specifically, we address the following questions: Is the plant-herbivore interaction driven (1) by the genotype of *Ambrosia* with some being resistant or at least highly unpalatable to all *Ophraella* genotypes? This would lead to a low and non-sustainable biocontrol efficacy after some years, when these plant genotypes become more abundant (scenario 1 in Fig. 1), (2) by the *Ophraella* genotype, leading to a highly efficient biocontrol management when using the most damaging insect genotypes (scenario 2 in Fig. 1) or (3) by specific *Ambrosia-Ophraella* genotype interactions (high G × G interaction (scenario 3 in Fig. 1), which would

elicit introducing multiple insect genotypes? To test which scenario best explains our findings, we performed a large full factorial *Ambrosia* genotype \times *Ophraella* genotype experiment. A significant *Ambrosia* and *Ophraella* effect will indicate scenarios 1 and 2, respectively, while a significant interaction term of the two-way analysis of variance for the studied response variates would point to scenario 3.

Material and methods

Study species

Ambrosia artemisiifolia (Asterales: Asteraceae), common ragweed, is an annual monoecious outcrossing plant native to North America, accidentally introduced in Asia, Australia and Europe, where it became an invasive alien species. It has significant negative effects on human health due to its highly allergenic pollen and on the yield in spring-sown crops, like sunflower and beets (Essl et al. 2015; Mouttet et al. 2018; Müller-Schärer et al. 2014; Schaffner et al. 2020).

The oligophagous leaf beetle *O. communis* has been used in China as a BCA since 2001, which is a natural enemy of *Ambrosia* in its native region in North America. It was accidentally introduced in Europe and first discovered in 2013 in Northern Italy and Southern Switzerland (Müller-Schärer et al. 2014). *Ophraella* development is composed of an egg stage, three larval instars (L1, L2 and L3 instar are used in the following text), a pupal stage and finally the adult stage (Suppl. material 1: Fig. S1; Zhou et al. 2010b). Larval and pupal development takes 7–17 days and 6–12 days, respectively (Lommen et al. 2017b; Zhou et al. 2010b). Each *Ophraella* stage can live and develop on *Ambrosia* (Yamazaki et al. 2000), with 4–5 generations in Northern Italy (Müller-Schärer et al. 2014), but up to 6–7 generations per year in southern China (Chen et al. 2013).

Sample collections

In order to use genotypes, we assumed to be genetically most distinct, we used *Ambrosia* seeds from 11 mother plants (half-sibs) from 11 regions in three continents (Table 1, Suppl. material 1: Fig. S2) and *Ophraella* from eight couples (full-sibs) from eight regions in three continents (Table 1, Suppl. material 1: Fig. S2). *Ambrosia* seeds were collected between 2013 and 2016 and conserved in paper envelopes in the cold chamber at the University of Fribourg (constant temperature: $\sim 5^\circ\text{C}$). *Ophraella* were collected in the field in 2017 and 2018 (cf. Table 1) and reared on *Ambrosia* plants grown from seeds of a mixture of Italian populations in the quarantine facility at the University of Fribourg ($22 \pm 5^\circ\text{C}$, 16:8 L:D cycle) for either two generations (Chinese locations) or one generation (all other locations) before their use in the experiment. This was done to reduce environmental maternal effects and to align the *Ophraella* development of the various collections with the phenology of the *Ambrosia* cohorts. The permit to import *A. artemisiifolia* and *O. communis* into our quarantine facility at the University of

Table 1. Origin of *Ambrosia artemisiifolia* seeds and *Ophraella communa* genotypes.

Species	Sample ID	Region	Continent	Coordinates	Date of collection
<i>Ambrosia</i>	USA-VA	Unionville	America	38.264968, -77.961216	2016-10-06
	USA-FL	Orlando	America	28.666826, -81.769223	2016-09-29
	China-ZX	Yongjiahezhen	Asia	31.146677, 114.709501	2013-10-14
	China-WH	Chengguanzhen	Asia	32.312290, 109.712106	2013-10-14
	Poland	Starzawa	Europa	49.877445, 23.013878	2016-10
	Croatia	Đurđanci	Europa	45.295130, 18.498682	2014-10
	Hungary	Tápiószentmárton	Europa	47.316969, 19.740684	2014-10
	France	Montceau-les-Mines	Europa	46.683864, 4.364136	2014-10-24
	Germany	Drebkau	Europa	51.666270, 14.231646	2014-09-30
	Romania	Văcărești	Europa	44.859988, 25.498338	2014-10-01
	Italy	Magnago	Europa	45.578542, 8.807434	2014-10-07
	Italy-MG	Magnago	Europa	45.580953, 8.793622	2018-06
<i>Ophraella</i>	USA-PA	North Belle Vernon	America	40.126039, -79.871702	2018-09
	USA-NY	Aurora	America	42.737028, -76.687861	2018-08
	USA-CN	Canon	America	40.455545, -78.429724	2018-09
	China-GX	Nanning	Asia	23.250000, 108.058000	2017-10
	China-HN	Linxiang	Asia	29.421000, 113.441000	2017-10
	Switzerland	Rovio	Europa	45.931040, 8.984031	2018-05
	Italy-LC	Lecco	Europa	45.826303, 9.355765	2018-06
	Italy-MG	Magnago	Europa	45.580953, 8.793622	2018-06

Fribourg was issued by the Swiss Federal Office for the Environment (permit number A130598-3).

To characterize the bioclimatic conditions of the sampled *Ambrosia* and *Ophraella* locations, interpolated GIS data were extracted for 19 climate factors from WorldClim collected over 30 years at 5 minutes spatial resolution (<https://www.worldclim.org/>). To illustrate eco-climatic variation among the different sampling regions, we performed a principal component analysis (PCA) and compared the similarity with a Mantel test, using 999 permutations and pairwise tests between organisms. Both sampled *Ambrosia* and *Ophraella* genotypes spread over a large eco-climatic range, but when the two species were superposed, the ellipses do not differ between the two species, indicating a high eco-climatic correspondence between the plant and herbivore populations sampled (Suppl. material 1: Fig. S3).

Experimental design and procedure

In 2018, seeds were germinated in Petri-dishes on double thickness moistened filter paper in the growing chamber (19 ± 5 °C, 14:10 L:D cycle). On the day of germination, seeds were transferred in trays filled with commercial soil (Proter + Pro type 4, Fenaco Genossenschaft, Switzerland, containing 150 mg/l N, 350 mg/l P₂O₅, 800 mg/l K₂O, salt content < 3 KCl et pH = 6.2 (CaCl₂) until they reached six leaves. Plants were then transplanted into 1 L pots filled with the commercial soil, vermiculite (Vermica AG, Bözen, Switzerland) and sand (2:2:1) and kept in the greenhouse of the University of Fribourg (25 ± 5 °C under a 16:8 L:D cycle). Every second day, new leaf (leaves > 5 mm long) pairs (nodes) were counted to account for leaf age when later used in

the experiment. In order to have leaves of the same age available (cf. below) for our tests, we repeatedly produced new cohorts of the plant genotypes. Plants were watered equally with 250 ml every second day.

Eight locations of *Ophraella* were reared on Italian *Ambrosia* plants in cages in the quarantine facility of the University of Fribourg. Each day, pupae from the cages were isolated in Petri-dishes on a clean filter paper. After emergence, several virgin *Ophraella* couples were randomly formed within each location. Each couple was isolated in Petri-dishes (Ø90 mm) with filter paper and fed with a fresh *Ambrosia* leaf from rearing plants by inserting the petiole in a wet floral foam. A single couple per location was selected for the experiment that laid enough eggs, to test full-siblings representing one *Ophraella* genotype (see Suppl. material 1: Fig. S4 for details). Each day, the selected couples were checked for new egg batches. Leaves with eggs were collected and isolated and a new leaf was added in the Petri-dish. Isolated egg batches were checked each day for egg hatching and L1 instar larvae were transferred with a paintbrush on the leaf from a specific *Ambrosia* genotype. Each larva was fed on 2 leaves (10–20 days old) during its development, the first leaf for L1 – L2 instar, and the second leaf for L3 instar to pupal instar, with both leaves coming from the same plant genotype. We opted for three replicates for all paired combinations of the 11 *Ambrosia* and 8 *Ophraella* genotypes by first transferring each one L1 instar larvae on three test leaves of the same plant genotype. In case an L1 instar died before developing to L2, which might have been caused by injuring the small larvae during the transfer on the leaves, we started a new test directly with a L2 instar larva from the same egg batch and a new leaf from the same plant individual. A few interactions could not be tested due to the lack of *Ambrosia* plants or *Ophraella* eggs. In total, we performed 233 tests by successfully transferring L1 and an additional 52 tests by transferring L2, resulting in a total of 285 tests.

Measurements

Larvae were checked daily for mortality and instar. Leaf area consumed during L2 and L3 larval instar was measured by comparing the leaf area before and after feeding. We scanned the leaf at the beginning and end of the L2 (leaf one) and at the beginning and end of L3 (leaf 2) using ImageJ software (v1.51k) to measure the leaf area. The difference between the two measurements equals the leaf area consumed by the larvae. The sex of newly emerged adults was determined with a binocular microscope and beetles were conserved in the freezer (-20 °C). Adult fresh weight and oven-dried weight (60 °C for 24 h) of each adult was measured using a Microbalance (Mettler MT-5, Mettler-Toledo, Inc., Columbus, OH, USA) with a resolution of 1 µg. The dry weight of each individual was then subtracted from its fresh weight to calculate the percentage of water (Zhou et al. 2011), as

$$\% \text{ water content} = \frac{\text{fresh weight} - \text{dry weight}}{\text{fresh weight}} .$$

Statistical analyses

We built 11×8 matrices representing *Ambrosia-Ophraella* genotype interactions for the different performance traits of *Ophraella* larvae and adults and for the leaf consumption, with x-axis for the *Ambrosia* and the y-axis for the *Ophraella* genotypes. Effects of plant and herbivore genotypes and their interactions on *Ophraella* performance were assessed using linear mixed-effects models and generalized linear mixed-effects models (LMM/GLMM) and fit using the *lmer/glmer* function obtained from the R package *lme4* that uses maximum likelihood to estimate the model parameters (Bates et al. 2014). In the models, the plant and the herbivore genotype and their interactions were included as fixed effects and leaf node level and sex (for adult biomass only) as random effects. Normality of the residuals of all models was assessed using QQ-plots. For the survival and the developmental time, GLMM with Binomial distribution and with Poisson distribution, respectively, was used; and LMM for leaf consumption, adult dry weight and adult water content. Mixed-effect regression models were used to analyze the correlation between dry biomass of *Ophraella* adults and total leaf area they consumed. Plant and herbivore genotype and their interactions were also included as fixed effects, leaf node level and sex as random effects. If a significant interaction term was detected, we analyzed each of the sex separately. We adjusted p-values using the Bonferroni–Holm method to correct for type 1 error. All statistical analyses were run with R version 3.6.1 (2019).

Results

Ophraella survival

We found significant effects of *Ophraella* genotypes on L1, L2 and L3 survival ($\chi^2 \geq 15.14$, $P_{adj} \leq 0.05$; Table 2), but not on pupal survival ($\chi^2 = 10.83$, $P_{adj} = 0.16$), whereas no significant effects were found for *Ambrosia* genotypes on L1, L2, L3 and pupal survival ($\chi^2 \leq 15.7$, $P_{adj} \geq 0.62$; Table 2). There were no significant effects of *Ambrosia-Ophraella* genotype interactions ($\chi^2 \leq 70.23$, $P_{adj} \geq 0.26$). In general, we found significant effects of *Ophraella* genotype ($\chi^2 = 26.20$, $p < 0.001$), but no effects of *Ambrosia* genotype and *Ambrosia-Ophraella* genotype interactions ($\chi^2 = 7.37$, $P_{adj} = 1$ and $\chi^2 = 65.92$, $P_{adj} = 0.47$; respectively) on adult emergence (Fig. 2); with the lowest emergence of *Ophraella* genotype from CN-GX (Fig. 2). European *Ophraella* genotypes (CH and Italy) had the best survival performance until adult, whereas no CN-GX larvae reached adult emergence (Table 2 and Suppl. material 1: Fig. S5).

Ophraella developmental time

There were significant effects of *Ophraella* genotype on developmental time of L2, L3 and pupae ($\chi^2 \geq 19.05$, $P_{adj} \leq 0.008$; Table 2), but not on L1 developmental

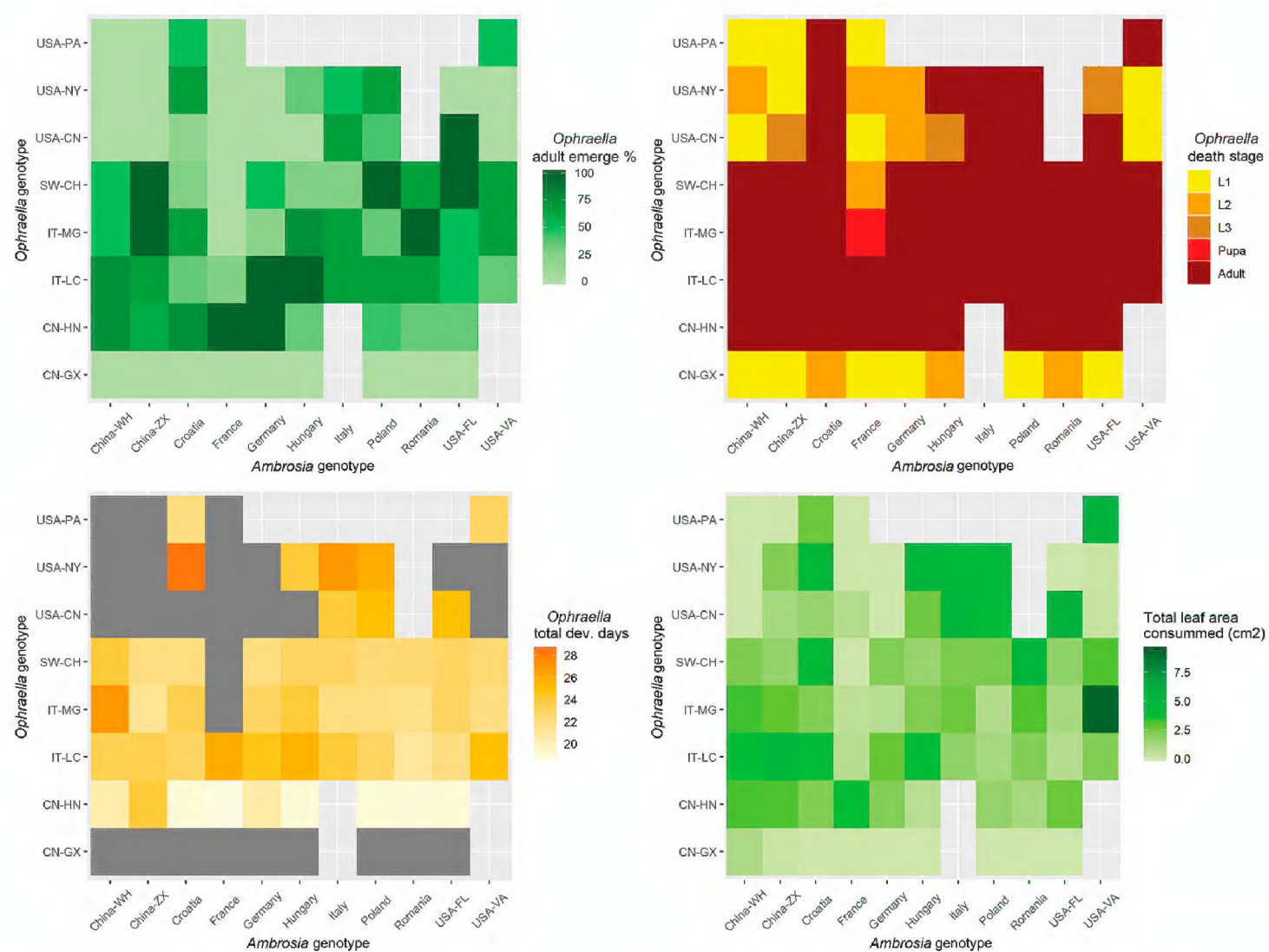


Figure 2. Effect of *Ambrosia artemisiifolia* and *Ophraella communa* genotype on *Ophraella* adult emergence (survival up to adults; up left), death stage (stage when died; up right), total developmental time (L1 to adult, gray cells indicate tests without adults emergence); bottom left), and on leaf area eaten during L2 and L3 instar (bottom right). Blank cells represent missing data. Dark-colored horizontal line means that the interaction is driven by the *Ophraella* genotype, dark-colored vertical line means that the interaction is driven by the *Ambrosia* genotype.

Table 2. Effects of *Ophraella communa* and *Ambrosia artemisiifolia* genotype and their interactions on herbivore performance and leaf area consumed. Bold *p*-values are statistically significant. ***: $P \leq 0.001$, **: $P \leq 0.01$, *: $P \leq 0.05$, .: $P \leq 0.1$, ns.: $P > 0.1$.

Measurement		<i>Ophraella</i>				<i>Ambrosia</i>				<i>Ophraella</i> × <i>Ambrosia</i>			
		χ^2	df	Adjusted <i>P</i> -value		χ^2	df	Adjusted <i>P</i> -value		χ^2	df	Adjusted <i>P</i> -value	
Survival	L1	71.91	7	<0.001	***	15.70	10	0.62	ns.	65.00	58	0.47	ns.
	L2	19.73	7	0.01	**	8.28	10	1	ns.	70.23	52	0.26	ns.
	L3	15.14	7	0.05	*	14.17	10	0.62	ns.	51.78	44	0.46	ns.
	Pupa	19.83	7	0.16	ns.	6.39	10	1	ns.	44.45	36	0.46	ns.
Adult emergence		26.20	7	<0.001	***	7.37	10	1	ns.	65.92	58	0.47	ns.
Developmental time	L1	4.17	7	0.76	ns.	3.49	10	1	ns.	17.55	43	1	ns.
	L2	19.05	7	0.01	**	7.24	10	1	ns.	28.11	44	1	ns.
	L3	37.77	7	<0.001	***	8.51	10	1	ns.	15.66	36	1	ns.
	Pupa	19.08	6	0.008	**	0.76	10	1	ns.	5.91	32	1	ns.
Total developmental time		13.29	6	0.05	*	1.40	10	1	ns.	4.29	32	1	ns.
Adult	Dry weight	36.10	7	<0.001	***	13.58	10	0.62	ns.	62.10	34	0.02	*
	% Water content	13.80	7	0.06	.	5.28	10	1	ns.	34.11	31	0.52	ns.
Total leaf area consumed		24.79	7	<0.001	***	29.58	10	0.01	**	43.99	31	0.26	ns.

time ($\chi^2 = 4.17$, $P_{adj} = 0.76$). *Ambrosia* genotype did not affect any developmental stage ($\chi^2 \leq 8.51$, $P_{adj} = 1$; Table 2), nor did the *Ambrosia-Ophraella* genotype interactions ($\chi^2 \leq 28.11$, $P_{adj} = 1$). In general, total developmental time in days from L1 instar till adult emergence showed a significant difference among *Ophraella* genotypes ($\chi^2 = 13.29$, $P_{adj} = 0.05$), but not among *Ambrosia* genotypes and for their interaction ($\chi^2 = 1.4$, $P_{adj} = 1$ and $\chi^2 = 4.29$, $P_{adj} = 1$; respectively) (Fig. 2), with the CN-HN genotype showing the fastest developmental time (Table 2 and Suppl. material 1: Fig. S6).

Leaf area consumed by *Ophraella* larvae

Both *Ophraella* and *Ambrosia* genotype affected the amount of leaf area eaten from L2 to pupae ($\chi^2 \geq 24.79$, $P_{adj} \leq 0.01$; Table 2, Fig. 2), without a significant interaction term ($\chi^2 = 43.99$, $P_{adj} = 0.26$; Table 2). CN-GX *Ophraella* genotype consumed the lowest and European *Ophraella* genotypes (CH and Italy) the largest amount of leaf area, and the *Ambrosia* genotypes from Germany and France were consumed the least (Suppl. material 1: Fig. S7).

Ophraella adult traits

We found significant effects of *Ophraella* genotype on adult dry weight ($\chi^2 = 36.10$, $P_{adj} < 0.001$) (Suppl. material 1: Fig. S7, Table 2) and marginally on water content ($\chi^2 = 13.80$, $P_{adj} = 0.06$), while no effect was found on these traits by *Ambrosia* genotype ($\chi^2 \leq 13.58$, $P_{adj} \geq 0.62$; Table 2). *Ambrosia-Ophraella* genotype interactions were significant for *Ophraella* dry weight ($\chi^2 = 62.10$, $P_{adj} = 0.02$), but not for water content ($\chi^2 = 34.11$, $P_{adj} = 0.52$; Suppl. material 1: Fig. S7). *Ophraella* females were significantly larger than males ($t = -3.765$, $P < 0.001$; Suppl. material 1: Fig. S8). We further found a significant correlation between beetle dry biomass and total *Ambrosia* leaf area consumed ($R^2 = 0.07$, $P = 0.004$; Suppl. material 1: Fig. S8).

Discussion

Ambrosia-Ophraella genotype interactions

We selected our *Ambrosia* and *Ophraella* genotypes from different continents and locations to reach a high genetic diversity for our tests. This genetic diversity also reflects the observed high within population genetic diversity found in European *Ambrosia* populations targeted for biocontrol (Genton et al. 2005; McGoey et al. 2019; van Boheemen et al. 2019). Our results of *Ophraella* performance provide strong evidence that the *Ophraella* genotype is driving the plant-herbivore interactions studied. We found significant effects of the different *Ophraella* genotypes on 11 of the 13 traits measured, including herbivore survival, developmental time, adult weight and food consumption. Higher survival of pupae than of larvae is in line with previous findings (Zhou et al.

2010a; Zhou et al. 2011). Moreover, seven traits were only significantly affected by *Ophraella* genotype including L1 and L3 survival, adult emergence, developmental time and relative water content of adults. Previous studies on *Ophraella* showed that a higher survival and faster developmental time would result in a higher population increase and more efficient control of *Ambrosia* (Augustinus et al. 2020). Furthermore, larger body size in insects is generally linked to greater fecundity and access to mates (Blanckenhorn 2000) and is thus expected to further enhance population build-up and expansion in *O. communis* (Chen et al. 2014). The *Ophraella* genotype from Linxiang, China (CN-HN) clearly showed the fastest developmental time on all *Ambrosia* genotypes, followed by the three European genotypes, which did slightly less well for this trait, but similarly well for survival and leaf area consumed. This might indicate that the studied three genotypes from Europe could be genetically closely related, such as when deriving from a single introduction event, but this needs to be further verified. Their overall good performance on most *Ambrosia* genotypes tested is so far a good sign for a successful biocontrol outcome in Europe.

With regard to outcomes for biocontrol management using *Ophraella* to suppress *Ambrosia* populations, our findings thus follow scenario 2 outlined in Fig. 1, indicating an optimal situation at least for a short- to mid-term biocontrol management. This result could be due to the shorter generation time of the antagonist as compared to its host plant, leading to a more virulent antagonist genotype (Kaltz et al. 1999), or due to its oligophagous nature, which allows *Ophraella* to deal with a large diversity of plant defense chemicals (Ali and Agrawal 2012). The fact that the *Ambrosia* genotype only differed for total leaf area consumed by the larvae, but did not influence survival and developmental time, or the weight of the herbivore, may indicate that *Ophraella* can compensate for observed differences in secondary plant metabolites in *Ambrosia* (Fukano and Yahara 2012; Sun and Roderick 2019; Wan et al. 2019) by adapting their feeding rate (Müller et al. 2006). Significant *Ambrosia*-*Ophraella* $G \times G$ interactions were found only for two out of 13 variates measured, i.e., L2 survival and dry weight of adults.

We are aware that our data on the beetle development and growth are considerably better than the data collected from the plant, i.e., that one effect was tested more thoroughly than the other. Our findings also might have been influenced by using cut leaves, known to elicit induced defense mechanisms (Beck et al. 2014), but this may hardly have changed our overall findings of the *Ophraella* genotype driving herbivore performance. Furthermore, we also acknowledge that there might be various trade-offs with larval performance that will affect the level of impact on the *Ambrosia* genotypes, such as host preference for oviposition, plant growth rate, regrowth capacity and tolerance (Zytynska and Preziosi 2011).

Scenarios of plant-antagonist genotype interactions in the context of a weed biocontrol project

In its introduced range, the level of genetic variation of a plant invader can vary from a single genotype as for *Rubus alceifolius* in La Reunion and Mauritius (Amsellem et

al. 2001) and location-specific haplotypes of the weed fern *Lygodium microphyllum* (Goolsby et al. 2006), to a few biotypes such as for the Asteracean *Chondrilla juncea* in Australia (Espiau et al. 1997; Gaskin et al. 2013) and up to a high within population genetic variation as found in *Lantana camara* in South Africa (Mukwevho et al. 2017) and *Ambrosia artemisiifolia* populations in Europe, where the within population variation even exceeds the level reported from their native range (van Boheemen et al. 2017). It would thus be helpful to know early in a biocontrol project, if a few genotypes or a single BCA biotype could efficiently control the target weed population, or whether multiple genotypes and populations of a BCA are needed, which would involve more time and money.

Based on these settings, we can distinguish three scenarios, with greatly different outcomes for a biocontrol management success (Fig. 1). The first scenario designates the situation where some specific plant genotypes are resistant or tolerant to all antagonist genotypes tested (Fig. 1; Underwood and Rausher 2000). In this case, the plant genotype drives the plant-antagonist interaction, such as when using insect- or pathogen-resistant/tolerant crop cultivars (Moreau et al. 2006; Scott et al. 2010). Genotypes of a plant invader in the introduced range may originate from an area with heavy attack by the BCA that was subsequently introduced to the same location. Such a long-evolved association may have resulted in a homeostatic relationship with little impact of the BCA (cf. Hokkanen and Pimentel 1989), but more recent evidence for such an outcome is yet missing. Furthermore, resistance or tolerance of the plant invader to the BCA could also arise through new, potentially transgressive genotypes resulting from admixtures after multiple introductions (e.g., for *A. artemisiifolia*, Genton et al. 2005; van Boheemen et al. 2017; for *Silene latifolia*, Wolfe et al. 2007), or through interspecific hybridization (e.g., for *Tamarix* spp., Gaskin and Kazmer 2009; and for *Fallopia* spp., Gammon et al. 2007; Krebs et al. 2010) in the introduced range. However, we are not aware of cases from the weed biocontrol literature that such genotypes of the plant invader were resistant or tolerant to introduced BCA, which leaves the scenario 1 presently rather theoretical. In the second scenario, the antagonist genotype drives the plant-antagonist interaction, i.e., when specific antagonist genotypes affect all or at least most of the plant genotypes, including the dominant ones (Fig.1; Lommen et al. 2017a; Roderick et al. 2012; Wajnberg 2004). This is expected to result in an unstable co-occurrence pattern with an effective and at least initially sustainable biocontrol (Lommen et al. 2017a). This scenario allows selecting genotypes or biotypes of more effective BCAs, which presently are mainly used in augmentative biocontrol (Lommen et al. 2017a; Szűcs et al. 2012). Thirdly, specific plant genotype by antagonist genotype interactions in a population are expected to result in an overall co-occurrence by maintaining the genetic diversity of both players (Sasaki 2000). In a biological management setting, this would entail the introduction of a suite of antagonist genotypes to reach overall control (Campanella et al. 2009; Goolsby et al. 2006).

The present study illustrates a conceptual approach on the G by G interaction between a BCA and its target plant by assessing whether the response variables are

better explained by the genetic variability of the BCA or the plant. Investigating the genetic structure both among and within populations of the plant invader and the BCA remain the important first steps in developing a successful weed biocontrol project. Should such investigations reveal distinct genetic variability, especially within the BCA, we advocate to integrate bioassays as outlined in this study during the pre-release phase of a biocontrol program. This will give a first indication of the probability for an at least short- to mid-term efficacy and sustainability when introducing a potential BCA, and on where to find the most efficient agent genotypes.

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Supplementary material I

Figure S1–S8

Authors: Yan Sun, Carine Beuchat, Heinz Müller-Schärer

Data type: image, occurrence, phenotypic data

Explanation note: **Figure S1.** Life cycle of the biocontrol candidate *Ophraella communa*. **Figure S2.** Origin of *Ambrosia artemisiifolia* seeds and *Ophraella communa* genotypes. **Figure S3.** Principal component analysis (PCA) of the *Ambrosia artemisiifolia* and *Ophraella communa* samples for 19 environmental factors. **Figure S4.** Set-up of the experimental design. **Figure S5.** Effect of all *Ambrosia artemisiifolia* and *Ophraella communa* genotypes on the survival of each larval instars and pupal stage of *O. communa*. **Figure S6.** Effect of all *Ambrosia artemisiifolia* and *Ophraella communa* genotypes on the developmental time of each larval instar and pupal stage of *O. communa*. **Figure S7.** Effect of all *Ambrosia artemisiifolia* and *Ophraella communa* genotypes on the dry weight and water content of *O. communa* emerged adults, and on the total, first and second leaf area consumed. **Figure S8.** Relationship between total leaf area consumed of *Ambrosia artemisiifolia* and *Ophraella communa* adult dry biomass, separately for sex.

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